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(54) Title: METHOD FOR DESTROYING RETINAL PIGMENT EPITHELIAL CELLS			
(57) Abstract A method for destroying retinal pigment epithelial cells in an eye in order to prevent the occurrence of proliferative vitreoretinopathy. A solution containing a basement membrane binding agent conjugated to a cytotoxic agent is introduced into the vitreous chamber. The solution is maintained in the vitreous chamber for a period of time sufficient to permit the basement membrane binding agent to bind to basement membranes lining the vitreous chamber. The solution is then removed from the vitreous chamber, whereby a portion of the basement membrane binding agent remains bonded to basement membranes within the vitreous chamber, thereby exposing retinal pigment epithelial cells disposed on the basement membrane to the cytotoxic agent.			

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METHOD FOR DESTROYING RETINAL PIGMENT EPITHELIAL CELLS

FIELD OF INVENTION

The present invention relates to a method for destroying retinal pigment epithelial and like proliferative cells for the purpose of preventing the occurrence of proliferative vitreoretinopathy (PVR) or traction band formation following the current treatment for retinal tears and detachments. More particularly, the present invention is directed to a method for destroying retinal pigment epithelial and like proliferative cells on the retina surface in the vitreous chamber of the eye through the application of a membrane-binding agent having a toxin bonded thereto.

BACKGROUND ART

Proliferative vitreoretinopathy (PVR) is the most common cause of blindness after retinal detachment. In order to gain access to a torn or detached retina, an incision typically is made at the pars plana of the eye for the purpose of introducing a surgical instrument into the vitreous chamber of the eye. In the case of retinal tears and detachments, a vitrectomy procedure and reattachment of the retina is performed in which a torn or detached portion of the retina is reattached using a surgical laser instrument, cautery, adhesives or tamponades. Following retinal detachment or tears, proliferative cells such as but not limited to retinal pigment epithelial cells, glial cells, fibroblasts, macrophages, myofibroblast-like cells

and the like are released into the vitreous chamber. These proliferative cells, thought to be primarily retinal pigment epithelial (RPE) cells and therefor hereinafter referred to collectively as RPE cells, then proliferate in the vitreous chamber. The proliferation of these cells in the vitreous chamber and on the retina, cause traction bands to form and re-detach the retina thus causing blindness. Accordingly, a method of preventing proliferative vitreoretinopathy is desired. A composition useful in the prevention of proliferative vitreoretinopathy is likewise desired.

DISCLOSURE OF INVENTION

The method of the present invention is used to destroy retinal pigment epithelial cells within the vitreous chamber of the eye following a retinal tear or detachment through the use of a basement membrane binding agent. A cytotoxic agent capable of destroying retinal pigment epithelial cells preferably is conjugated to the basement membrane binding agent. Following vitrectomy, if necessary, and retina reattachment, the basement membrane binding agent conjugated to the cytotoxic agent is introduced into the vitreous chamber of the eye. The basement membrane binding agent conjugated to the cytotoxic agent is allowed to remain in the vitreous chamber for a predetermined period of time sufficient to permit the basement membrane binding agent to bind to the basement membrane lining the vitreous chamber of the eye, and more specifically, the inner limiting membrane (ILM). Any excess material is then removed from the vitreous chamber. Retinal pigment epithelial cells released into the

vitreous chamber and subsequently disposed on the basement membranes are thus exposed to the cytotoxic agent conjugated to the basement membrane binding agent, resulting in the destruction of the retinal pigment epithelial cells.

5 In an alternative embodiment of the method of the present invention preferably for use in aphakic eyes, a first basement membrane binding agent is introduced into the eye prior to performing a vitrectomy. The first basement membrane binding agent is allowed to remain in the eye for a
10 predetermined period of time sufficient to permit the basement membrane binding agent to bind to the basement membranes within the eye. Excess of the first basement membrane binding agent is then removed from the eye. A vitrectomy, if necessary, and retina reattachment is then
15 performed, thus exposing additional basement membranes within the vitreous chamber. A second basement membrane binding agent having a cytotoxic agent conjugated thereto is then introduced into the eye and permitted to remain therein for a period of time sufficient to permit the basement
20 membrane binding agent to bind to the basement membranes within the vitreous chamber. Any excess material is then removed from the eye. Retinal pigment epithelial cells disposed on the basement membranes within the vitreous chamber are thus exposed to the cytotoxic agent conjugated
25 to the second basement membrane binding agent, resulting in the destruction of the retinal pigment epithelial cells.

MODE(S) FOR CARRYING OUT THE INVENTION

The method of the present invention is intended to destroy proliferative cells referred to herein collectively as retinal pigment epithelial cells disposed on the interior surfaces of the vitreous chamber of the eye. By destroying these cells, the cells can not proliferate and/or migrate along the surface of the retina and form the traction bands which are indicative of proliferative vitreoretinopathy (PVR) resulting in retinal detachment. The subject method thereby is capable of preventing the occurrence of proliferative vitreoretinopathy (PVR). The method of the present invention can be employed in connection with any retina reattachment procedure.

Vitreotomy and retina reattachment entails the formation of an incision through a surface of the eye in order to provide direct access to the internal vitreous chamber of the eye. Although the necessary incision is usually formed at the pars plana of the eye, it will be appreciated that alternative locations for this incision can be selected at the discretion of the surgeon. Following the formation of the incision, a vitrectomy is usually required and performed prior to introducing a cautery instrument through the incision into the vitreous chamber of the eye. The instrument usually a laser, is advanced through the vitreous chamber such that the laser or like suitable instrument is in direct contact with the retina surface of the eye. The retina is reattached through the use of the laser for cauterization thereof. Alternatively, the retina

may be reattached using suitable adhesives and/or tamponades.

It has been discovered that certain agents will bind to basement membranes, including basement membranes within the vitreous chamber. The basement membrane binding agents, when bonded to the basement membrane or ILM of the vitreous chamber, are in direct contact with the retinal pigment epithelial cells during the progression of PVR. The method of the present invention is based upon observations of such cellular proliferation.

In a first embodiment of the method of the present invention, a solution containing a basement membrane binding agent is introduced into the vitreous chamber following vitrectomy if performed and retina reattachment. In this first embodiment of the present invention, a cytotoxic agent is conjugated to the basement membrane binding agent. As above-discussed, the basement membrane binding agent will bind to the basement membranes that form the interior surface of the vitreous chamber, thereby providing direct contact between retinal pigment epithelial cells and the cytotoxic agent conjugated to the basement membrane binding agent. The solution containing basement membrane binding agent conjugated to the cytotoxic agent is retained within the vitreous chamber for a predetermined period of time sufficient to permit the basement membrane binding agent to bind to the basement membranes within the vitreous chamber. It will be appreciated that the length of time required for binding the basement membrane binding agent to the basement membranes within the vitreous chamber will be dependent upon

a number of factors, including, but not limited to, the concentration of the basement membrane binding agent in the solution that is introduced into the vitreous chamber.

Excess of the solution containing the basement membrane
5 binding agent conjugated to the cytotoxic agent is then removed from the vitreous chamber. A variety of techniques can be used for the removal of this solution, including known aspiration and irrigation/aspiration techniques.

Following removal of the excess solution containing the
10 basement membrane binding agent from the vitreous chamber of the eye, a tamponade may or may not be applied and the incision in the eye is then closed to complete the procedure.

It will be appreciated that the basement membrane
15 binding agent will remain bonded to the basement membranes within the vitreous chamber. The cytotoxic agent conjugated to the basement membrane binding agent will thus have direct access to the retinal pigment epithelial cells on the interior surface of the vitreous chamber. In particular, it
20 will be appreciated that the retinal pigment epithelial cells will come into contact with the basement membrane binding agent as the retinal pigment epithelial cells attempt to proliferate and migrate across the interior surface of the vitreous chamber. The retinal pigment
25 epithelial cells will internalize the cytotoxic agent, thereby resulting in the destruction of the retinal pigment epithelial cells on the interior surface of the vitreous chamber and thus preventing PVR and the resultant blindness.

In an aphakic eye, it should be noted that the anterior chamber of the eye is exposed to the vitreous chamber of the eye and also includes basement membranes, e.g., the corneal endothelium. Due to the fact that corneal endothelium will not regenerate once it has been damaged, particular care should be exercised to ensure that the solution containing the basement membrane binding agent conjugated to a cytotoxic agent does not come into contact with the corneal endothelium. A second embodiment of the present invention is intended to protect the corneal endothelium from the effects of the cytotoxic agent.

In the second embodiment of the method of the present invention, a first solution containing a basement membrane binding agent without a cytotoxic agent conjugated thereto is introduced into the eye prior to performing a vitrectomy and retina reattachment. The basement membrane binding agent in the first solution will bind to the exposed basement membranes within the eye, particularly the corneal endothelium. The first solution containing a basement membrane binding agent is allowed to remain in the eye for a predetermined period of time sufficient to ensure that adequate bonding has occurred between the first basement membrane binding agent and the basement membranes within the eye. The first solution containing a basement membrane binding agent is then removed from the eye using known aspiration or irrigation/aspiration methods as above-discussed with respect to the first embodiment of the present invention. A vitrectomy if necessary and retina

reattachment procedure are then performed using known techniques.

A second solution containing a basement membrane binding agent conjugated to a cytotoxic agent is then
5 introduced into the vitreous chamber. It is preferable that the second solution bind only to those basement membranes within the eye not previously bonded to the first basement membrane binding agent, thereby ensuring that the cytotoxic agent destroys retinal pigment epithelial cells within the
10 vitreous chamber without damaging the corneal endothelia. For this reason, it is preferable that the first basement membrane binding agent be the same substance as the second basement membrane binding agent, thus ensuring that the first and second basement membrane binding agents bind to
15 the same sites on the basement membranes.

Polylysine can be used as a basement membrane binding agent in connection with the method of the present invention. It will be appreciated that the molecular weight of polylysine or other polymeric basement membrane binding
20 agents can be varied. This aspect of polymeric basement membrane binding agents makes then particularly beneficial when used in connection with the method of the present invention. In particular, it will be appreciated that the binding affinity of a polymeric basement membrane binding
25 agent such as polylysine will increase as its molecular weight increases due to the increased number of binding sites available on the higher molecular weight molecule. Higher molecular weight polylysine molecules thus will tend to displace lower molecular weight polylysine molecules

previously bonded to a basement membrane. For the above-discussed reasons, if a polymeric basement membrane binding agent such as polylysine is used in connection with the method of the present invention, it is preferable that the molecular weight of the first basement membrane binding agent be at least as great as the molecular weight of the second basement membrane binding agent in order to ensure that the second basement membrane binding agent does not displace the first basement membrane binding agent. In order to provide even greater assurances that the second basement membrane binding agent does not displace the first basement membrane binding agent, it is preferably that the molecular weight of the first basement membrane binding agent be greater than the molecular weight of the second basement membrane binding agent.

The second solution containing a basement membrane binding agent conjugated to a cytotoxic agent is retained within the vitreous chamber for a predetermined period of time sufficient to ensure that adequate bonding has occurred between the second basement membrane binding agent and the basement membranes not previously bonded to the first basement membrane binding agent. As above-discussed, the amount of time required is dependent upon a number of factors. Excess of the second solution is then removed from the eye using known techniques such as aspiration or irrigation/aspiration. For the reasons discussed above with respect to the first embodiment of the present invention, the cytotoxic agent will tend to destroy retinal pigment

epithelial cells within the vitreous chamber, thereby preventing PVR.

In a third embodiment of the present invention, the above-described steps of the first embodiment of the invention are performed. Next, a second solution containing a second basement membrane binding agent is provided and is introduced into the eye for the purpose of displacing any of the first basement membrane binding agent that may have come into contact with the corneal endothelium. For the above-discussed reasons, it is preferable that the second basement membrane binding agent be the same substance as the first basement membrane binding agent. In addition, it is preferable that the molecular weight of the second basement membrane binding agent be greater than the molecular weight of the first basement membrane binding agent, thereby ensuring that the second basement membrane binding agent will displace the first basement membrane binding agent on the corneal endothelium.

A variety of basement membrane binding agents can be used in connection with the method of the present invention. In a preferred embodiment of the present invention polylysine is employed as a basement membrane binding agent. Poly-L-lysine has been shown to be effective when used in connection with the method of the present invention. It is possible that poly-D-lysine also can be effective. Polylysine is advantageously used in connection with the present invention due to its availability, relatively low cost, and its ability to be formulated in a variety of molecular weights. Other basement membrane binding agents

believed to be useful in connection with the present invention includes, but are not limited to, fibronectin, fibroblast growth factors, laminin, type IV collagen, perlecan, decorin, thrombospondin, tenascin, vitronectin, 5 heparin, heparan sulfate, poly-arginine, dextran, dextran sulfate, chondroitin sulfate, hyaluronic acid, platelet factor IV, fibrin, and fibrinogen.

A variety of known cytotoxic agents can be used in conjunction with the method of the present invention. In a 10 preferred embodiment of the present invention, a ribosomal inhibitory protein such as saporin or ricin is used as the cytotoxin agent. Ribosomal inhibitory proteins are preferable due to the fact that they contain more inhibitory activity per microgram than other cytotoxic agents that can 15 be used in connection with the method of the present invention. Other cytotoxic agents believed to be efficacious when used in connection with the method of the present invention include, but are not limited to, antimitotic drugs such as methotrexate, 5-fluorouracil, daunomycin, 20 doxorubicin, mitoxanthrone, vinca alkaloids, vinblastine, colchicine, or cytochasins, when used as the cytotoxic agent. In addition, ionophores such as monensin and ouabain can be used as the cytotoxic agent in connection with the method of the present invention. It will be appreciated that 25 antimitotic conjugates will destroy retinal pigment epithelial cells while exhibiting less toxicity to the iris and corneal endothelium compared to the ribosomal inhibitory proteins.

A variety of known methods can be employed for conjugating the cytotoxin to the basement membrane binding agent. For example, the carboxyl groups of the cytotoxic agent can be bonded to the amines of the basement membrane binding agent using a water-soluble carbodiimide technique. When this technique for conjugation is used, the entire conjugate will be internalized by the retinal pigment epithelial cells and the basement membrane binding agent will then be degraded by the cell to release the cytotoxic agent.

Hetero-bi-functional cross-linkers such as SPDP also can be used to conjugate the cytotoxic agent to the basement membrane binding agent, thereby creating a disulfide bond between the cytotoxic agent and the basement membrane binding agent. Once the resulting hybrid molecule is internalized by the retinal pigment epithelial cell, the disulfide bond is hydrolyzed to release the cytotoxic agent, thereby resulting in the destruction of the cell.

It is believed that recombinant DNA technology also can be used to construct the gene for a toxin with a basement membrane binding protein sequence incorporated therein. This gene may be expressed in a host cell and the product purified from the growth medium.

By way of example and not by way of limitation, a conjugate of polylysine and saporin was prepared by coupling polylysine to SPDP in accordance with the instructions stated by the manufacturer of the SPDP. The free SPDP was then removed using a filtration technique or, in the alternative, through the use of a sepharose heparin column.

The resulting polylysine-SPDP was then reduced with dithiothreitol. Saporin was then coupled with SPDP in the same manner and added to the solution of polylysine-SPDP. The resulting solution filtered to remove uncoupled agents, thereby producing a conjugated polylysine-saporin solution.

The solution containing the basement membrane binding agent can comprise a variety of known biocompatible agents. In one embodiment of the present invention, the basement membrane binding agent is contained in a balanced salt solution. Solutions containing viscoelastics such as methylcellulose also can be used in connection with the present invention. It will be appreciated that other known biocompatible solutions can be used in connection with the method of the present invention.

Although the present invention has been described herein with respect to certain preferred embodiments, it will be appreciated by one of ordinary skill in the pertinent art that various modifications can be made without departing from the intended spirit and scope of the invention.

CLAIMS

1. A method for destroying retinal pigment epithelial cells in a vitreous chamber of an eye following vitrectomy and retina reattachment, said method comprising:

5 providing a first solution containing a first basement membrane binding agent conjugated to a cytotoxic agent;

introducing said first solution into said vitreous chamber;

maintaining said first solution in said vitreous
10 chamber for a predetermined period of time sufficient for said first basement membrane binding agent to bind to a basement membrane within said vitreous chamber; and

removing said first solution from said vitreous chamber, whereby retinal pigment epithelial cells on said
15 basement membrane within said vitreous chamber are exposed to said first basement membrane binding agent conjugated to a cytotoxic agent such that said retinal pigment epithelial cells internalize said first basement membrane binding agent conjugated to a cytotoxic agent, and whereby said retinal
20 pigment epithelial cells are destroyed by said cytotoxic agent.

2. A method for destroying retinal pigment epithelial cells in a vitreous chamber of an eye following vitrectomy and retina reattachment in accordance with claim 1, wherein
25 said first basement membrane binding agent is selected from a group consisting of: polylysine, fibronectin, laminin, type IV collagen, perlecan, decorin, thrombospondin, tenascin, vitronectin, heparin, heparan sulfate, poly-

arginine, dextran, dextran sulfate, chondroitin sulfate, hyaluronic acid, platelet factor IV, fibrin, and fibrinogen.

3. A method for destroying retinal pigment epithelial cells in a vitreous chamber following vitrectomy and retina reattachment in accordance with claim 2, wherein said first basement membrane binding agent is polylysine.

4. A method for destroying retinal pigment epithelial cells in a vitreous chamber following vitrectomy and retina reattachment in accordance with claim 1, wherein said cytotoxic agent is selected from a group consisting of: saporin, ricin, methotrexate, 5-fluorouracil, daunomycin, doxorubicin, mitoxanthrone, vinca alkaloids, vinblastine, colchicine, cytochasin monensin, and ouabain.

5. A method of destroying retinal pigment epithelial cells in a vitreous chamber following vitrectomy and retina reattachment in accordance with claim 4, wherein said cytotoxic agent is selected from a group consisting of saporin and ricin.

6. A method for destroying retinal pigment epithelial cells in a vitreous chamber following vitrectomy and retina reattachment in accordance with claim 4, wherein said cytotoxic agent is saporin.

7. A method for destroying retinal pigment epithelial cells in a vitreous chamber following vitrectomy and retina

reattachment in accordance with claim 1, wherein said first solution comprises a balanced salt solution.

8. A method for destroying retinal pigment epithelial cells in a vitreous chamber following vitrectomy and retina
5 reattachment in accordance with claim 1, wherein said first solution comprises a viscoelastic material.

9. A method for destroying retinal pigment epithelial cells in a vitreous chamber following vitrectomy and retina reattachment in accordance with claim 1, wherein said
10 cytotoxic agent is conjugated to said first basement membrane binding agent using a water soluble carbodiimide technique.

10. A method for destroying retinal pigment epithelial cells in a vitreous chamber following vitrectomy and retina
15 reattachment in accordance with claim 1, wherein said cytotoxic agent is conjugated to said first basement membrane binding agent using a hetero-bi-functional cross-linker.

11. A method for destroying retinal pigment epithelial
20 cells in a vitreous chamber following vitrectomy and retina reattachment in accordance with claim 1, wherein said cytotoxic agent is conjugated to said first basement membrane binding agent is polylysine and wherein said cytotoxic agent is saporin.

12. A method for destroying retinal pigment epithelial cells in a vitreous chamber following vitrectomy and retina reattachment in accordance with claim 1, wherein said method further comprises:

5 providing a second solution containing a second basement membrane binding agent, said second basement membrane binding agent having a molecular weight greater than a molecular weight of said first basement membrane binding agent;

10 introducing said second solution onto corneal endothelial basement membranes within a vitreous chamber of said eye following removal of said first solution from said eye, whereby said second basement membrane binding agent displaces said first basement membrane binding agent to the
15 extent that any of said first basement membrane binding agent has bonded to said corneal endothelial basement membranes, thereby preventing damage to said corneal endothelial basement membranes; and
 removing said second solution from said eye.

20 13. A method for destroying retinal pigment epithelial cells in a vitreous chamber following vitrectomy and retina reattachment in accordance with claim 12, wherein said first basement membrane binding agent and said second basement membrane binding agent are polylysine.

25 14. A method for destroying retinal pigment epithelial cells in a vitreous chamber of an eye, said method comprising:

providing a first solution containing a first basement membrane binding agent, said first basement membrane binding agent having a molecular weight;

introducing said first solution into said eye prior to
5 performing vitrectomy and retina reattachment;

maintaining said first solution in said eye for a predetermined period of time sufficient for said first basement membrane binding agent to bind to a basement membrane within a vitreous chamber of said eye;

10 removing said first solution from said eye;

performing a vitrectomy and retina reattachment;

providing a second solution containing a second basement membrane binding agent conjugated to a cytotoxic agent, said second basement membrane binding agent having a
15 molecular weight;

introducing said second solution into said vitreous chamber;

maintaining said second solution in said vitreous chamber for a predetermined period of time sufficient for
20 said second basement membrane binding agent to bind to a basement membrane within said vitreous chamber; and

removing said second solution from said vitreous chamber, whereby retinal pigment epithelial cells on said basement membrane within said vitreous chamber are exposed
25 to said second basement membrane binding agent conjugated to a cytotoxic agent such that said retinal pigment epithelial cells internalize said second basement membrane binding agent conjugated to a cytotoxic agent, and whereby said

retinal pigment epithelial cells are destroyed by said cytotoxic agent.

15. A method for destroying retinal pigment epithelial cells in a vitreous chamber of an eye in accordance with claim 14, wherein said first basement membrane binding agent and said second basement membrane binding agent are selected from a group consisting of: polylysine, fibronectin, laminin, type IV collagen, perlecan, decorin, thrombospondin, tenascin, vitronectin, heparin, heparan sulfate, polyarginine, dextran, dextran sulfate, chondroitin sulfate, hyaluronic acid, platelet factor IV, fibrin, and fibrinogen.

16. A method for destroying retinal pigment epithelial cells in a vitreous chamber in accordance with claim 15, wherein said first basement membrane binding agent and said second basement membrane binding agent are polylysine.

17. A method for destroying retinal pigment epithelial cells in a vitreous chamber in accordance with claim 16, wherein said molecular weight of said first basement membrane binding agent is greater than said molecular weight of said second basement membrane binding agent.

18. A method for destroying retinal pigment epithelial cells in a vitreous chamber in accordance with claim 14, wherein said cytotoxic agent is selected from a group consisting of: saporin, ricin, methotrexate, 5-fluorouracil,

daunomycin, doxorubicin, mitoxanthrone, vinca alkaloids, vinblastine, colchicine, cytochasin monensin, and ouabain.

19. A method for destroying retinal pigment epithelial cells in a vitreous chamber in accordance with claim 18, wherein said cytotoxic agent is selected from a group consisting of saporin and ricin.

20. A method for destroying retinal pigment epithelial cells in a vitreous chamber in accordance with claim 18, wherein said cytotoxic agent is saporin.

22. A method for destroying retinal pigment epithelial cells in a vitreous chamber in accordance with claim 14, wherein said first solution and said second solution comprise a balanced salt solution.

23. A method for destroying retinal pigment epithelial cells in a vitreous chamber in accordance with claim 14, wherein said cytotoxic agent is conjugated to said second basement membrane binding agent using a water soluble carbodiimide technique.

24. A method for destroying retinal pigment epithelial cells in a vitreous chamber in accordance with claim 14, wherein said cytotoxic agent is conjugated to said second basement membrane binding agent using a hetero-bi-functional cross-linker.

25. A method for destroying retinal pigment epithelial cells in a vitreous chamber in accordance with claim 14, wherein said second basement membrane binding agent is polylysine and wherein said cytotoxic agent is saporin.

5 26. A composition for destroying retinal pigment epithelial cells in a vitreous chamber comprising a basement membrane binding agent conjugated to a cytotoxic agent.

27. A composition for destroying retinal pigment epithelial cells in a vitreous chamber comprising polylysine
10 conjugated to a cytotoxic agent.

28. A composition for destroying retinal pigment epithelial cells in a vitreous chamber in accordance with claim 26 or 27, wherein said cytotoxic agent is selected from a group consisting of saporin and ricin.

15 29. A composition for destroying retinal pigment epithelial cells in a vitreous chamber in accordance with claim 26 or 27, wherein said cytotoxic agent is saporin.

30. A method for destroying proliferative cells in a vitreous chamber of an eye following retina reattachment,
20 said method comprising:

 providing a first solution containing a first basement membrane binding agent conjugated to a cytotoxic agent;

 introducing said first solution into said vitreous chamber;

maintaining said first solution in said vitreous chamber for a predetermined period of time sufficient for said first basement membrane binding agent to bind to a basement membrane within said vitreous chamber; and

5 removing said first solution from said vitreous chamber, whereby proliferative cells on said basement membrane within said vitreous chamber are exposed to said first basement membrane binding agent conjugated to a cytotoxic agent such that said proliferative cells
10 internalize said first basement membrane binding agent conjugated to a cytotoxic agent, and whereby said proliferative cells are destroyed by said cytotoxic agent.

31. A method for destroying proliferative cells in a vitreous chamber of an eye following retina reattachment in
15 accordance with claim 30, wherein said first basement membrane binding agent is selected from a group consisting of: polylysine, fibronectin, laminin, type IV collagen, perlecan, decorin, thrombospondin, tenascin, vitronectin, heparin, heparan sulfate, poly-arginine, dextran, dextran
20 sulfate, chondroitin sulfate, hyaluronic acid, platelet factor IV, fibrin, and fibrinogen.

32. A method for destroying proliferative cells in a vitreous chamber following retina reattachment in accordance with claim 31, wherein said first basement membrane binding
25 agent is polylysine.

33. A method for destroying proliferative cells in a vitreous chamber following retina reattachment in accordance with claim 30, wherein said cytotoxic agent is selected from a group consisting of: saporin, ricin, methotrexate, 5-fluorouracil, daunomycin, doxorubicin, mitoxanthrone, vinca alkaloids, vinblastine, colchicine, cytochasins monensin, and ouabain.

34. A method of destroying proliferative cells in a vitreous chamber following vitrectomy and retina reattachment in accordance with claim 33, wherein said cytotoxic agent is selected from a group consisting of saporin and ricin.

35. A method for destroying proliferative cells in a vitreous chamber following retina reattachment in accordance with claim 33, wherein said cytotoxic agent is saporin.

36. A method for destroying proliferative cells in a vitreous chamber following retina reattachment in accordance with claim 30, wherein said first solution comprises a balanced salt solution.

37. A method for destroying proliferative cells in a vitreous chamber following retina reattachment in accordance with claim 30, wherein said first solution comprises a viscoelastic material.

38. A method for destroying proliferative cells in a vitreous chamber following retina reattachment in accordance with claim 30, wherein said cytotoxic agent is conjugated to said first basement membrane binding agent using a water
5 soluble carbodiimide technique.

39. A method for destroying proliferative cells in a vitreous chamber following retina reattachment in accordance with claim 30, wherein said cytotoxic agent is conjugated to said first basement membrane binding agent using a hetero-
10 bi-functional cross-linker.

40. A method for destroying proliferative cells in a vitreous chamber following retina reattachment in accordance with claim 30, wherein said cytotoxic agent is conjugated to said first basement membrane binding agent is polylysine and
15 wherein said cytotoxic agent is saporin.

41. A method for destroying proliferative cells in a vitreous chamber following retina reattachment in accordance with claim 30, wherein said method further comprises:

providing a second solution containing a second
20 basement membrane binding agent, said second basement membrane binding agent having a molecular weight greater than a molecular weight of said first basement membrane binding agent;

introducing said second solution onto corneal
25 endothelial basement membranes within a vitreous chamber of said eye following removal of said first solution from said

eye, whereby said second basement membrane binding agent displaces said first basement membrane binding agent to the extent that any of said first basement membrane binding agent has bonded to said corneal endothelial basement membranes, thereby preventing damage to said corneal endothelial basement membranes; and
5 removing said second solution from said eye.

42. A method for destroying proliferative cells in a vitreous chamber following retina reattachment in accordance with claim 41, wherein said first basement membrane binding agent and said second basement membrane binding agent are polylysine.
10

43. A method for destroying proliferative cells in a vitreous chamber of an eye, said method comprising:
15 providing a first solution containing a first basement membrane binding agent, said first basement membrane binding agent having a molecular weight;
introducing said first solution into said eye prior to performing retina reattachment;
20 maintaining said first solution in said eye for a predetermined period of time sufficient for said first basement membrane binding agent to bind to a basement membrane within a vitreous chamber of said eye;
removing said first solution from said eye;
25 performing a retina reattachment;
providing a second solution containing a second basement membrane binding agent conjugated to a cytotoxic

agent, said second basement membrane binding agent having a molecular weight;

introducing said second solution into said vitreous chamber;

5 maintaining said second solution in said vitreous chamber for a predetermined period of time sufficient for said second basement membrane binding agent to bind to a basement membrane within said vitreous chamber; and

removing said second solution from said vitreous
10 chamber, whereby proliferative cells on said basement membrane within said vitreous chamber are exposed to said second basement membrane binding agent conjugated to a cytotoxic agent such that said proliferative cells
internalize said second basement membrane binding agent
15 conjugated to a cytotoxic agent, and whereby said proliferative cells are destroyed by said cytotoxic agent.

44. A method for destroying proliferative cells in a vitreous chamber of an eye in accordance with claim 43, wherein said first basement membrane binding agent and said
20 second basement membrane binding agent are selected from a group consisting of: polylysine, fibronectin, laminin, type IV collagen, perlecan, decorin, thrombospondin, tenascin, vitronectin, heparin, heparan sulfate, polyarginine, dextran, dextran sulfate, chondroitin sulfate, hyaluronic
25 acid, platelet factor IV, fibrin, and fibrinogen.

45. A method for destroying proliferative cells in a vitreous chamber in accordance with claim 44, wherein said

first basement membrane binding agent and said second basement membrane binding agent are polylysine.

46. A method for destroying proliferative cells in a vitreous chamber in accordance with claim 45, wherein said
5 molecular weight of said first basement membrane binding agent is greater than said molecular weight of said second basement membrane binding agent.

47. A method for destroying proliferative cells in a vitreous chamber in accordance with claim 43, wherein said
10 cytotoxic agent is selected from a group consisting of: saporin, ricin, methotrexate, 5-fluorouracil, daunomycin, doxorubicin, mitoxanthrone, vinca alkaloids, vinblastine, colchicine, cytochasins monensin, and ouabain.

48. A method for destroying proliferative cells in a
15 vitreous chamber in accordance with claim 47, wherein said cytotoxic agent is selected from a group consisting of saporin and ricin.

49. A method for destroying proliferative cells in a vitreous chamber in accordance with claim 47, wherein said
20 cytotoxic agent is saporin.

50. A method for destroying proliferative cells in a vitreous chamber in accordance with claim 43, wherein said first solution and said second solution comprise a balanced salt solution.

51. A method for destroying proliferative cells in a vitreous chamber in accordance with claim 43, wherein said cytotoxic agent is conjugated to said second basement membrane binding agent using a water soluble carbodiimide
5 technique.

52. A method for destroying proliferative cells in a vitreous chamber in accordance with claim 43, wherein said cytotoxic agent is conjugated to said second basement membrane binding agent using a hetero-bi-functional cross-
10 linker.

53. A method for destroying proliferative cells in a vitreous chamber in accordance with claim 43, wherein said second basement membrane binding agent is polylysine and wherein said cytotoxic agent is saporin.

15 54. A composition for destroying proliferative cells in a vitreous chamber comprising a basement membrane binding agent conjugated to a cytotoxic agent.

55. A composition for destroying proliferative cells in a vitreous chamber comprising polylysine conjugated to a
20 cytotoxic agent.

56. A composition for destroying proliferative cells in a vitreous chamber in accordance with claim 54 or 55, wherein said cytotoxic agent is selected from a group consisting of saporin and ricin.

57. A composition for destroying proliferative cells in a vitreous chamber in accordance with claim 54 or 55, wherein said cytotoxic agent is saporin.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/01408

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/00

US CL : 514/2, 8, 12, 21, 23

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 8, 12, 21, 23

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONEElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,920,104 A (DEVORE ET AL.) 24 April 1990, see entire document.	1-57

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

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